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Award Number: W81XWH-09-1-0398

TITLE: Targeted Approaches to Overcoming Endocrine Resistance in Breast Cancer

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REPORT DATE: August 2010

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

√ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-08-2010	Annual Summary	1 AUG 2009 - 31 JUL 2010

4. TITLE AND SUBTITLE

Targeted Approaches to Overcoming Endocrine Resistance in Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0398

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Illinois

Urbana IL 61801-3620

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Fort Detrick MD 21702-5014

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

None

14. ABSTRACT

The research outlined in this proposal is aimed directly at improving the effectiveness and duration of endocrine therapies.
Our approach builds upon our initial observations that tamoxifen up-regulates 14-3-3z, a key scaffold protein that is associated with poor outcome of patients on tamoxifen endocrine therapy. 14-3-3z interacts with and enhances the activity of growth factor receptors and kinases that are overexpressed in breast cancers that are resistant to endocrine therapies. The experiments outlined are aimed at validating 14-3-3z as a marker for risk of recurrence due to the development of endocrine resistance and at establishing this protein as a target whose inhibition would enhance the effectiveness of endocrine therapies, by maintaining endocrine sensitivity. Thus, the outcome of this research has the potential for improving the selection of breast cancer patients most likely to benefit from endocrine therapies and provide a new avenue for enhancing the effectiveness of these therapies for treatment of breast cancer.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	11	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

Endocrine therapies (antiestrogens such as tamoxifen or aromatase inhibitors such as letrozole) initially benefit many of the ca. 75% of breast cancers that are estrogen receptor positive. The effectiveness of endocrine therapies, however, is often lost with time because the tumor cells become resistant. We have recently found that loss of benefit from endocrine therapy involves increased activity of protein kinases and growth factors that work along with the protein 14-3-3ζ(zeta) to promote survival of the tumor cells. Their studies indicate that high levels of the 14-3-3ζ protein are found in breast cancers that show a poor clinical outcome on endocrine therapy. These findings imply that targeting 14-3-3ζ might prove useful for enhancing and prolonging the effectiveness of endocrine therapies.

Body

Despite the benefits of estrogen receptor (ER) targeted endocrine therapies in women with hormone-sensitive breast cancers, a significant proportion unfortunately experience disease progression, due to the acquisition of resistance to the therapy. 14-3-3ζ, a member of the 14-3-3 family of highly conserved proteins, is present on a region of recurrent genomic gain (chromosome 8q22), and is overexpressed in many breast cancers. We previously reported that tamoxifen stimulated increased 14-3-3ζ expression in ER-positive breast cancer cells and that high expression of 14-3-3ζ in breast tumors was associated with a poor clinical outcome for women on endocrine therapy.

We have now found that reducing cellular levels of 14-3-3ζ markedly increases apoptosis of breast cancer cells, reduces cell proliferation and motility, decreases receptor tyrosine kinase signaling and, importantly, reverses antiestrogen resistance, thereby rendering endocrine-resistant breast cancer cells sensitive to antiestrogens. As expected, these beneficial effects of 14-3-3ζ depletion were lost upon re-expression of 14-3-3ζ [A1, MS1].

Further, in four independent breast cancer microarray data sets from over 400 women, we found that high levels of 14-3-3ζ were associated predominantly with the ER-positive HER2 expressing luminal B subtype of breast cancers, and with a poor prognosis. Further, high expression of 14-3-3ζ correlated strongly with over-expression of genes functioning in mitosis and cytokinesis, including Aurora Kinase B, Polo Kinase 1, BIRC5 (survivin), and FOXM1. The latter four proteins were significantly decreased upon reduction of cellular 14-3-3ζ, suggesting their coregulation [A1, MS1].

Our findings emphasize the major detrimental role of 14-3-3ζ in contributing to endocrine resistance and to tumor progression, through regulation of key proteins in mitosis and cytokinesis. Thus, 14-3-3ζ is a cell survival and anti-apoptotic factor that increases breast cancer aggressiveness and likelihood of recurrence (proliferation, migration, and invasiveness). Our findings imply a primary role for 14-3-3ζ in promoting breast cancer growth and aggressiveness, and endocrine resistance. Hence, we believe that the development of therapies targeting 14-3-3ζ should prove valuable in maintaining or restoring endocrine sensitivity in breast cancer, and reducing risk of disease recurrence.

Our recent studies have also shown that ER activity, which promotes cancer cell proliferation and progression, depends on both nuclear-initiated and extranuclear-initiated estrogen receptor signaling pathways. These pathways involve the activation of protein kinases by estrogen receptors and collaborating growth factor receptors, such as HER2 and EGFR, and our studies have demonstrated that these kinase pathways are highly upregulated in endocrine resistance. Because 14-3-3ζ promotes protein kinase signaling by binding to phosphoserine and phosphothreonine motifs in these important signaling proteins, we believe that 14-3-3ζ may serve as a critical link in integrating inputs from the ER and protein kinase pathways in hormone-resistant breast cancers [A2, A3].

We have begun to examine 14-3-3ζ levels and intracellular localization in matched tissue arrays of the primary breast cancer prior to endocrine therapies and from the same patient after treatment at the time of recurrence. We are monitoring the intensity of 14-3-3ζ protein immunohistochemical staining and its nuclear/cytoplasmic/membrane distribution, and we correlated these with tumor aggressiveness and time to recurrence. We found that aggressive metastatic breast cancers showed higher 14-3-3ζ protein levels, with alterations in intracellular localization, compared to more indolent breast cancers. Moreover, site of recurrence seemed to be correlated with 14-3-3ζ level, and that high 14-3-3ζ expression is associated with distant recurrence. Further, we found a significant membrane-localized 14-3-3ζ staining in HER2 overexpressing tumors. Since upregulation and dysregulation of these receptor tyrosine kinase activities are known to be associated with endocrine-resistant breast tumor, we believe that 14-3-3ζ serves as a critical integrator, linking estrogen receptor and growth factor receptor-protein kinase actions [MS 2].

Below is listed the approved Statement of Work for year 1 reporting the status of the proposed tasks.

Statement of Work

	Specific Aim #1 – ***Characterize the role of 14-3-3z in ER-mediated and growth factor-mediated proliferation and suppression of apoptosis of breast cancer cells***	***Resources***	***Accomplishments***
Year 1-2	**Task 1a[months 1-6]:** Generation of 14-3-3z tetracycline-regulated construct (knockdown and overexpression) cell lines **Task 1b [months 7-10]:** Functional characterization of stable cell lines by proliferation, apoptosis, invasion and three- dimensional culture assays **Task 1c [months 11-24]:** SERMs, fulvestrant, aromatase inhibitor dose studies and starting tumor xenograft work *All tasks(1 through 3) will be performed in Benita Katzenellenbogen laboratory at University of Illinois*	• Cell lines used: MCF-7, MCF-7/Her2, MCF-7 Tam^R [tamoxifen resistant], MCF-7/Aromatase [aromatase overexpressing], ZR75 [ER positive, moderate Her2 and EGFR], BT474 [high Her2, low ER], MCF-10A [non-tumorigenic, ER negative] • Animal use: 120 nude mice will be included and equally divided into control and treatment groups (SERMs,Fulvestrant AI- three doses each) of 6 animals per group for wild type and 14-3-3z knockdown cells	**Tasks 1a –b: COMPLETED** **Task 1c**: on going

Milestones: 3 high impact publications and meeting presentation (AACR or San Antonio Breast Cancer Meeting)			We are currently working on 2 high impact manuscripts
	Specific Aim #2 – ***Examination of 14-3-3z levels and subcellular distribution in human breast tumors as a marker of tumor aggressiveness and endocrine responsiveness***	***Resources***	
Year 1-2	**Task 2a [months 12-16]**: Evaluation of 14-3-3z levels by immunohistochemical staining of breast tumor **specimens** **Task 2.b [months 17-20]:** Perform breast tumor tissue microarray **Task 2.c [months 20-24]:** Data analysis from Task 1c xenograft work	• 40 matched breast tumors from Elizabeth Wiley, University of Illinois Medical School in Chicago • BR701 tissue array from US Biomax	**Task 2a-b: COMPLETED** **Task 2c**: on going

Key Research Accomplishments

In the first year of this study funded by DoD we were able to identify that:

1. High levels of 14-3-3ζ were associated predominantly with the ER-positive HER2 expressing luminal B subtype of breast cancers, and with a poor prognosis.
2. High expression of 14-3-3ζ correlated strongly with over-expression of genes functioning in mitosis and cytokinesis
3. Reducing cellular levels of 14-3-3ζ markedly increases apoptosis of breast cancer cells, reduces cell proliferation and motility, decreases receptor tyrosine kinase signaling and, importantly, reverses antiestrogen resistance.
4. Aggressive metastatic breast cancers show higher 14-3-3ζ protein levels, with alterations in intracellular localization, compared to more indolent breast cancers

Reportable Outcomes

Published Abstracts from this project:

A1. **Bergamaschi, A., Frasor, J., and Katzenellenbogen, B.S**., A Gene Signature and Molecular Phenotype Associated with High Expression of 14-3-3ζ and Its Correlation with Antiestrogen Resistance in Breast Cancer. 2nd Jensen Symposium on Nuclear Receptors, University of Cincinnati Medical Center, Cincinnati, OH, October 2009.

A2. **Katzenellenbogen, B.S., Madak-Erdogan, Z., Bergamaschi, A., Stossi, F., Lupien, M., Brown, M., Katzenellenbogen, J.A.** Genomics of Estrogen Receptor Signaling in Breast Cancer and Endocrine Resistance. Keystone Symposium on Nuclear Receptors: Signaling, Gene Regulation and Cancer/Nuclear Receptors: Development, Physiology and Disease, Keystone, Colorado, March, 2010

A3. **Katzenellenbogen, B.S., Madak-Erdogan, Z., Bergamaschi, A., Stossi, F., Charn, T.H.** Genomics of Estrogen Receptor Signaling in Target Cells. Frontiers in Estrogens, SERMs, and TSEC Scientific Meeting, Philadelphia, PA, April, 2010

Manuscript in preparation from this project:

MS 1. **Bergamaschi, A., Frasor, J., Christensen, B.L., and Katzenellenbogen, B.S.,** Gene Signature and Molecular Phenotype Associated with High Expression of 14-3-3ζ in Breast Cancer and Reversal of Endocrine Therapy Resistance by 14-3-3ζ Down-Regulation/Depletion/Reduction

MS 2. **Bergamaschi, A., Stanculescu, A., Borgen K., Wiley E., Frasor, J., and Katzenellenbogen, B.S.,** Identification of 14-3-3z as a Critical Predictor of Breast Cancer Metastasis.

Conclusion

Our studies indicate that 14-3-3ζ is a major contributor to endocrine resistance and to breast cancer aggressiveness and recurrence. Our proposed studies should clarify the role of 14-3-3ζ by analysis of tumor tissue microarrays and characterization of gene expression signatures. We will also explore several therapeutic strategies to block the actions of 14-3-3ζ or reduce its levels, so as to maintain or restore endocrine sensitivity in breast cancer, thereby making endocrine therapies more effective for many breast cancer patients.

References

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A1. **Bergamaschi, A., Frasor, J., and Katzenellenbogen, B.S**., A Gene Signature and Molecular Phenotype Associated with High Expression of 14-3-3ζ and Its Correlation with Antiestrogen Resistance in Breast Cancer. 2nd Jensen Symposium on Nuclear Receptors, University of Cincinnati Medical Center, Cincinnati, OH, October 2009.

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MS 2. **Bergamaschi, A., Stanculescu, A., Borgen K., Wiley E., Frasor, J., and Katzenellenbogen, B.S.,** Identification of 14-3-3z as a Molecular Target of Metastatic Breast Carcinoma